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A PRELIMINARY REPORT ON THE YEARLY ORIGIN
AND DISSEMINATION OF PUCCINIA
GRAMINIS

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(WITH PLATE IV)

Introduction

The annual reappearance of *Puccinia graminis* Pers., the black rust of cereals, and its dissemination among the various species of the Gramineae, have long remained obstinate problems. For nearly a century botanists and also agronomists, because of its economic importance, have endeavored to discover how this fungus passes the winter and spreads to the grain fields. A review of the literature, to bring out certain points which have been too much overlooked by those who are committed to the conception that the barberry is the sole source of spring infection, will be of interest in connection with the data which my own observations afford.

The barberry was considered a disseminator of rust by careful observers centuries ago. Even as early as 1660, as a result of their reports, an act of Parliament was passed at Rouen (22) condemning the use of this shrub in the vicinity of grain fields. A similar law was passed in Mass. (28) in 1755. Successive measures of this nature were subsequently enacted throughout Europe (3).

Proof of the influence of the barberry on rust contagion was furnished by SCHÖLER (29),¹ who in 1818 succeeded in infecting

¹ Cited by ERIKSSON.

rye with aecidiospores from barberry leaves. SCHÖLER's publication, however, remained buried until brought to light by NIELSON.

Infection of the barberry with *P. graminis* was not accomplished until 1865, when DE BARY (3) readily infected the young leaves with germinating sporidia, thus establishing the heteroecism of the organism. Unfortunately he was unable at the same time to germinate the mature aecidiospores, either fresh or after various periods of preservation, and hence the cereal host species was not reinfected, although the following year he accomplished this upon young rye plants.

The distance rust is spread from the barberry is variously estimated by different writers. MARSHALL (24),² who set large barberry bushes in the grain fields and made careful observations, states that the rust extended 10 yards in the direction of the prevailing wind. SCHÖLER (29), on the other hand, used small bushes and found that it scattered over an area of 30 to 40 square feet. Much greater distances are frequently recorded in the literature, although usually based upon casual observation or opinion. WITHERING (33),³ for instance, advises that no barberry bushes should be planted within 300-400 yards of a grain field.

The absence of the barberry and *Mahonia* in several regions where *Puccinia graminis* is prevalent, as cited for Ecuador by LAGERHEIM (21), seems to indicate that the heteroecism of the fungus is merely facultative. According to BARCLAY (1), *P. graminis* is also commonly present in Jeypore, India, while the nearest barberry bushes are nearly 300 English miles distant. Moreover, "the aecidia are formed in summer, while the wheat and barley are grown in winter and harvested in April or May." In this same category ZUKAL (34) places Bosnia and Herzegovina, where, according to BRANDIS (34), the aecidial hosts, if present at all, are very rare. Perhaps the most striking case of this kind is in Australia, where *P. graminis* causes enormous damage to wheat, yet the barberry is not present and the aecidial stage has never been found.

The existence of a perennial mycelium, although established for a

² Cited by ARTHUR, Bull. Torr. Bot. Club 31:113. 1904.

³ Cited by ERIKSSON and HENNING (16).

number of rusts, has never been proved for *P. graminis*. ERIKSSON (13, 14, 16) found the mycelium scarcely extending beyond the contour of the pustules. DE BARY held that the development of the rust begins anew each year by the "germination of the teleutospores alone."

P. graminis was formerly considered one species capable of infecting various members of the Gramineae, and this supposedly wide range of infecting power was thought to conduce to its spread. ERIKSSON (16), however, after extensive inoculation experiments in Sweden, divides it into the following biological forms, which he finally classes as species:

Puccinia graminis secalis (*Secale cereale*, *Hordeum vulgare*, and *Triticum repens*).

Puccinia graminis avenae (*Avena sativa*).

Puccinia graminis tritici (*Triticum vulgare*).

Puccinia graminis airae (*Aira caespitosa*).

Puccinia graminis poae (*Poa compressa*).

Puccinia phlei-pratensis (*Phleum pratense*).

CARLETON'S (10) experiments in America, however, do not support ERIKSSON and HENNING'S results. He finds no distinction between the forms on wheat and barley. His results appear to establish two things: (1) "that the forms of black stem rust on wheat and barley, *Hordeum jubatum*, *Agropyron tenerum*, *A. Richardsoni*, and *Elymus canadensis glaucifolius*, are identical, with the probability that those on *Elymus virginicus muticus* and *Holcus lanatus* should be included; (2) that the black stem rust of *Agropyron occidentale* is physiologically distinct from any other."

Direct inoculation of the gramineous host with the germinating teleutospores has received some attention, although not as much as it deserves. Foremost among these experiments are probably those of DE BARY, who showed that the germinating sporidia of *P. graminis* would not infect the leaves of *Triticum vulgare*, *T. repens*, and *Avena fatua*. THÜMEN (31), however, asserts that the sporidia of *Melampsora salicina* infect the willow quite as easily as do the uredospores. PLOWRIGHT (27) also, in a detailed report, claims to have infected wheat plants directly with sporidia (teleuto-bearing straw), although he afterward informed KLEBAHN that

“his work rested upon an error.” Other failures to infect wheat with germinating sporidia are reported by WARD (32) and ERIKSSON (15), though no mention is made of the number of trials nor the point of inoculation. Notwithstanding these failures, BREFFELD (8) thinks further attempts should be made to infect young cereals with germinating teleutospores. His discovery that only the youngest tissues of cereals are penetrated by smut sporelings gives encouragement for numerous experiments in this direction. The hardened tissues may offer too much resistance to the delicate germ tubes of the sporidia, he says, which bore directly through the epidermis instead of entering through the stomata, as do the germinating uredospores and aecidiospores.

The behavior of the germinating teleutospores is influenced somewhat by their environment. MAGNUS (23) found that teleutospores of *P. graminis* kept under a thin layer of water formed a germ tube instead of a promycelium. These results were afterward confirmed by BLACKMAN (5), who also included two other genera. Other factors are sometimes operative, as KIENITZGERLOFF (18) reports that thin-walled teleutospores of *Gymnosporangium clavariaeforme* also form a germ tube. When teleutospores germinate in air, however, they almost invariably form sporidia. This is true of *P. graminis* even in Australia, where it has no aecidial host species.

The uredospores of *P. graminis* soon lose their viability, according to DE BARY (3), in one to two months if kept dry. BOLLEY (6, 7), however, obtained a germination of 5 per cent after exposing them to air and sunlight during the month of August, and even claims they may live over winter. Christman (11) failed to germinate them after November 23, although the uredospores of *P. coronata* were viable the 26th of January, and those of *P. rubigovera* the 21st of March. Perhaps the most extensive experiments of this kind have been made by ERIKSSON and HENNING (16), whose results show that the uredospores of both *P. graminis* and *P. glumarum* are unable to survive the winter in Sweden. The absence of fresh uredo pustules during two or three months in the spring is cited as additional proof, since the period of incubation after inoculation with uredospores is only about ten days. The

statement is also made by KLEBAHN (19) that "in Germany *P. graminis* does not appear to pass the winter as uredo." CARLETON (9) found this to be true in Kansas, and in a two weeks' trip through Texas in December 1895, he could find no rust on winter wheat or oats, although it was present there in abundance the previous summer.

The mycelium of the rust is not limited to the leaves and culms of cereals, but also enters the seed. ERIKSSON (14, 16) found it in abundance in the "peripheral tissues" of grains, but was unable to trace it into the young seedlings. ZUKAL (34) has made similar discoveries, and even found it in the seed coats of barley which was furnished by ERIKSSON and supposed to contain mycoplasma.

Spores of the Uredineae are frequently present in the seed of the host. SMITH (30) found teleutospores of *P. graminis* in oat grains lying next to the gluten layer, and ERIKSSON and HENNING (16) report the presence of both uredospores and teleutospores of *P. glumarum* in the pericarp of cereal grains.

Several rusts have been shown to infect their host species through the seed. CARLETON (10) demonstrated this conclusively for the *Euphorbia* rust (*Uromyces euphorbiae* C and P) with the seeds of *Euphorbia dentata*. The plants grown from disinfected seed were always free from rust, while the controls were heavily infected, although planted in sterilized soil and protected by bell jars. MASSEE (25) states that *P. malvacearum* Mont. commonly enters young hollyhock plants through the seed. According to MCALPINE (26) *P. beckmanniae* was introduced into Australia in 1903 and *P. impatientis* in 1904 through seed of *Beckmannia erucaeformis* and *Elymus condensatus*, respectively, received from the U.S. Department of Agriculture.

Experimental evidence seems to indicate that rusts may infect cereals through the seed. When oats were introduced into Ecuador by growing European seed in the botanical garden at Quito, LAGERHEIM (21) reports that the plants became heavily infected with *P. coronifera*, although neither this rust nor any of its aecidial hosts had ever been found in Ecuador. Carefully planned experiments, covering a period of years, were conducted by ERIKSSON (15) to determine whether *P. glumarum* winters in the seed of wheat

and barley. Glass cages of various sizes and types, provided with cotton ventilators to filter the air and caps to keep out the rain, were employed. Some were attached to a suction pump and artificially ventilated; others, on all sides except the north, were fitted with double panes of glass, between which was passed a continuous current of water. Despite all these efforts to produce a normal environment for the plants, the air inside the cages was always 2–6° degrees hotter than the air outside, and the light considerably diminished. The plants were always abnormal, often attaining two or three times the height of their outside neighbors. In a few cases a single winter cereal plant was placed in each of several glass tubes early in the spring, long before any rust appeared outside. As a rule, however, seeds were planted in pots of sterilized soil and placed in the bottom of the cages. As all the air entering the cages passed through cotton filters, no spores could be carried in from the outside. Although the majority of his results were negative, a considerable number of infections was obtained with both winter cereals and annuals. This he considered fully as much as could be expected, since the plants were grown under abnormal conditions. A similar set of experiments was planned by MASSEE (25), who planted wheat seed infected with *P. rubigovera* in two pots of soil and kept them covered with bell jars provided with cotton wool filters. In one pot 26 per cent of the plants rusted, and in the other 47 per cent, while not a pustule appeared on the controls.

The amount of rust developing in the grain field seems to vary somewhat with the date of sowing. Both the early and late grain, according to ERIKSSON and HENNING'S tables for the different cereals, are usually less rusty than those sown at an intermediate date. GALLOWAY (17) called attention to this fact in 1893, when all his duplicate plots of grain, sowed ten days later than the originals, were free from rust. Moreover, he says, "Examining the weather records for ten days preceding the rust, we find nothing to warrant the belief that the simultaneous appearance of the fungus the first week in May in widely separated spots was due to peculiar climatic conditions."

ERIKSSON'S well known mycoplasma theory was advanced to

explain such cases. This theory is based chiefly upon the claim that rust apparently infects the cereal host through the seed, although its mycelium is not recognizable in the germ by aid of the microscope and present cytological technique. KLEBAHN (20), for a time at least, supported this view, and even figured the nuclei of the fungus while in the mycoplasmic condition.

The following experiments on the life history of *P. graminis* were made at the North Dakota Agricultural College and Experiment Station at Fargo, North Dakota, to obtain information which would aid in producing a rust epidemic yearly in our wheat breeding plots to test the strains selected for rust resistance. This is a spring wheat region, where the winters are exceedingly cold and consequently winter cereals are not grown.

The germinating rusted wheat grains were studied at Madison, Wisconsin, under the direction of Dr. R. A. HARPER.

Method

All the plants used for inoculation, except when otherwise stated, were grown in the greenhouse in beds containing 6-8 inches of fertile soil. The grass plants were transplanted to pots and placed in the greenhouse several weeks before they were used. The temperature was kept reasonably cool, and the air fairly moist by frequently watering the floor.

The inoculations were made by first mixing the spores of a pustule in a small quantity of distilled water. The plants were then moistened with distilled water by means of an atomizer and the spores applied with a camel's hair brush. Except in a few cases where the plants were very large, they were kept under bell jars 24-48 hours. If they became dry, they were sprayed again the second day. Generally, however, they were quite moist when the covers were removed. For part of the work inverted test tubes attached to stakes at suitable heights were employed, the lower end being lightly closed with cotton wool. The test tubes were found to retain the moisture even better than the bell jars. Parallel marks were sometimes made on the leaves by means of India ink and the spores placed between them. The

beds containing these plants were generally watered just before making the inoculations, to provide a source of moisture for the air beneath the covers. When not stated, the height of the plants on which bell jars or test tubes were used varied from 8 to 13 inches, without straightening up the leaves.

The wheat seedlings, grown from the rusted seed at Madison, Wis., and sectioned for study, were killed and fixed in Flemming's strong solution, imbedded in paraffin, and stained with either iron-alum hematoxylin or the triple stain.

Experimental investigations

Experiments were begun to show how readily sporidia of *P. graminis* from wheat and native grasses infect the barberry, although there are very few barberry bushes in North Dakota, so few in fact that they could hardly be considered as a source of rust epidemics, unless miraculous powers were attributed to the wind in causing a widespread and uniform distribution of aecidiospores. In the following experiments small pieces of dead straw, which were covered with teleutospores of *P. graminis* and had lain on the ground during the winter, were arranged parallel to each other and tied longitudinally on the branches of the barberry bushes (*Berberis vulgaris*), just as the buds were beginning to unfold in the spring.

TABLE I

INOCULATION EXPERIMENTS WITH TELEUTOSPORES OF *Puccinia graminis* Pers.

Number of experiment	Date of inoculation	Source of material	Number of branches inoculated	Results
5.....	April 30, 1906	Agr. ten.*	5	5 positive
6.....	April 30, 1906	Agr. ten.	7	1 positive
7.....	April 30, 1906	Wheat	2	2 positive
9.....	April 30, 1906	Agr. rep.	2	2 positive
10.....	April 30, 1906	Wheat	1	1 positive
11.....	April 30, 1906	Hord. jub.	2	2 positive
12.....	April 30, 1906	Ely. trit.	2	1 positive
13.....	April 30, 1906	Wheat	1	1 positive
14.....	May 2, 1906	Wheat	3	3 negative

* Abbreviations: Agr. ten. = *Agropyron tenerum*; Agr. rep. = *Agropyron repens*; Hord. jub. = *Hordeum jubatum*; Ely. trit. = *Elymus triticoides*; Ber. vul. = *Berberis vulgaris*.

In experiments 5-13 inclusive, the inoculated barberry branches were heavily infected the 14th of May. Only spermatogonia were present on this date, but they were frequently on both surfaces of the leaves. There were a few spermatogonia on other parts of the bushes, but no such dense blotches as appeared where the rusty straw was applied.

Further inoculations were made upon the barberry by scraping the teleutospores from dead straw, wintered the same as that described for Table I, and placing it on young buds or leaves which were carefully marked off by India ink and tagged. These results are recorded in the following table.

TABLE II

INOCULATIONS MADE UPON *Berberis vulgaris* WITH TELEUTOSPORES FROM *Puccinia graminis* Pers.

Number of experiment	Date of inoculation	Source of material	Number of parts inoculated	Results
19.....	May 8, 1906	Oats	10 buds	10 negative
22.....	May 9, 1906	Ely. trit.	10 buds	2 positive
27.....	May 21, 1906	Ber. vul.	10 buds	10 negative
28.....	May 22, 1906	Wheat	8 leaves	8 positive
52.....	June 26, 1906	Ely. trit.	1 leaf	negative
53.....	June 26, 1906	Hord. jub.	1 leaf	negative

As shown by the table, these results were mainly negative, perhaps on account of poor germination, since the buds selected were fully as young as those used earlier.

In order to obtain pure cultures of aecidiospores of known origin for further experiments relative to their infecting power, inoculations were made upon barberry bushes which had been standing in the greenhouse in large tubs for nearly a year and had borne no rust. The infections were made by tying small pieces of old rusty straw on the bushes in places offering the least opportunity for contamination and moistening the straw frequently with distilled water. A summary of the results is shown in Table III.

Aecidia appeared only on parts of the barberry inoculated, and, with the exception of the rusty oat straw from which all my inoculations so far have failed, infections were obtained from each kind of material. This with the data of the two preceding

tables shows that under favorable conditions *P. graminis* passes readily to the barberry from wheat and certain grasses, viz., *Agropyron tenerum*, *A. repens*, *Hordeum jubatum*, and *Elymus triticoides*, confirming the general results of DE BARY and others.

TABLE III

INOCULATIONS MADE UPON *Berberis vulgaris* WITH TELEUTOSPORES OF *Puccinia graminis* Pers.

Number of experiment	Date of inoculation	Source of material	Method	Results
1.....	March 29, 1907	Agr. ten.	Bell jar	Positive
2.....	March 29, 1907	Agr. ten.	Bell jar	Negative
3.....	March 29, 1907	Agr. rep.	Bell jar	Positive
4.....	March 29, 1907	Agr. rep.	Bell jar	Negative
5.....	March 29, 1907	Oats	Bell jar	Negative
6.....	March 29, 1907	Oats	Bell jar	Negative
7.....	March 29, 1907	Oats	Bell jar	Negative
8.....	March 30, 1907	Wheat	Bell jar	Positive
9.....	March 30, 1907	Wheat	Uncovered	Negative
10.....	March 30, 1907	Hord. jub.	Bell jar	Positive
11.....	March 30, 1907	Hord. jub.	Uncovered	Negative
12.....	April 4, 1907	Wheat	Uncovered	Negative
13.....	April 4, 1907	Wheat	Uncovered	Negative

Observations were made on the dissemination of rust from barberry bushes by taking note of the infection on the surrounding grasses. There was a small barberry hedge in Fargo very favorably located for this purpose, as it was surrounded on three sides by meadow and was heavily rusted every year. Careful observations for three successive springs (1905-1907) furnished some surprising data. Early each year, the plants of *Hordeum jubatum*, *Agropyron repens*, and *A. tenerum* in the immediate vicinity of the hedge became thoroughly covered with the uredo stage, while *Phleum pratense* and *Poa serotina* were absolutely free from it, and *Elymus virginicus* bore only an occasional pustule. The rust was abundant within 25 yards of the barberry bushes, but practically disappeared at a distance of 60 yards. The most persistent searching was required to discover a single pustule beyond 80 yards, and in no one of the three springs at this early date, before rust had begun to spread from the uredospores, could I find fresh uredo pustules of *P. graminis* beyond 100 yards from the barberry hedge, notwithstanding the fact that in 1905 rust was fairly abun-

dant in this region. Either the aecidiospores are not borne as great distances by the wind as formerly supposed, or their germinative power is remarkably low. There is some additional evidence in support of the latter view.

Plots of small wheat plants in the experimental garden were sprayed repeatedly with aecidiospores in the spring of 1905, yet scarcely any rust appeared until the plants were nearly two feet high, a fact commonly observed here in the field every spring, although volunteer wheat plants barely out of the ground in the fall are often covered with rust.

Two series of infection experiments were made to obtain further data with reference to the spreading of *P. graminis* by means of its aecidiospores. From 98 aecidial pustules, taken at random in 1906, a total of 368 plants were inoculated. Plants of wheat, rye, oats, barley, and usually *Avena fatua*, *Agropyron tenerum*, *A. repens*, and *Hordeum jubatum* were inoculated from each aecidial pustule and covered with bell jars 24–48 hours. Germination tests of the spores, made by placing them in water and on wet filter paper kept in a moist chamber at 18–20° C., showed a viability of about 8 per cent. Rust appeared only on *Avena fatua*, *Agropyron repens*, rye, oats, and *Hordeum jubatum*. No plants of barley or wheat were infected. These experiments were repeated in 1907 by inoculating 247 plants from 13 pustules of known origin, the original host species always being included in each group. Tests of the spores showed about the same percentage of germination as those used in the former experiments. No barley plants were infected, and the only wheat plants which developed rust were those inoculated with a form which came originally from wheat. The aecidiospores of only 9 pustules, however, of the 111 used in the two series of experiments caused infection. This relatively low number of infections agrees with the results obtained by repeatedly spraying the wheat plots with aecidiospores in 1905, and may partly account for the confining of the rust to the immediate vicinity of the barberry hedge as observed for the three successive years 1905–1907. It is also in harmony with the very limited spreading observed by both MARSHALL and SCHÖLER when they set barberry bushes in the

grain fields, and might easily be accounted for by the change of host species if the heteroecism of the fungus is only facultative.

A suspicion has frequently been expressed that the black rust spreads to the grain fields by aid of the grasses which either harbor the mycelium over winter or are infected early by aecidiospores. In order to determine the interval between the appearance of rust on grasses and cereals, the following observations were made in the spring of 1905. Rust the following summer, although not as abundant in North Dakota as in 1904, was still quite pronounced.

TABLE IV

FIRST APPEARANCE OF *Puccinia graminis* Pers. UPON GRASSES AND CEREALS AT
FARGO, NORTH DAKOTA, IN THE SPRING OF 1905

Date of observation	Host species	Spore pustules	Location	Remarks
June 27 . .	Hord. jub.	Uredo	Grass garden	A few pustules on a single plant
June 29 . .	Hord. jub.	Uredo	Near barberry	Far removed from barberry bushes.
July 6 . . .	Spring wheat	Uredo	Field	
July 9 . . .	Agr. rep.	Uredo	Near barberry	Present in abundance on both Agr. rep. and Hord. jub. None found elsewhere although a diligent search was made.
July 10 . .	Winter wheat*	Uredo	Field	Found in abundance at a considerable distance from barberry bushes.
July 12 . .	Agr. rep.	Teleuto	Near barberry	Present on both Agr. rep. and Hord. jub.
July 13 . .	Winter wheat	Teleuto	Field	Same plot of winter wheat mentioned above.
July 16 . .	Spring wheat	Uredo	Field	Appearing quite generally on all the oldest wheat.

* This was an experimental plot of winter wheat in charge of Dean J. H. SHEPPERD, the agronomist, and the writer was not aware of its presence until July 10, when the plants which had survived the winter were thoroughly covered with mature uredo pustules of *P. graminis*, some quite old, the rust having first appeared probably 10-14 days earlier.

The foregoing table shows that *P. graminis* probably appeared upon the experimental plot of winter wheat almost or quite as early as upon *Agropyron repens* and *Hordeum jubatum*, even when the latter were in the immediate vicinity of the barberry. It also shows that, with the exception of the one case mentioned

under date of June 27, the uredospores of *P. graminis* were generally present upon the spring wheat earlier than they were observed upon the wild grasses remote from the barberry bushes. In fact, *P. graminis* was present in the uredo stage upon spring wheat July 6, and with one exception could not be found upon the grasses remote from the barberry even July 9, after which date no further search was made for uredo upon the latter.

Experiments were made to obtain data with reference to the spread of *P. graminis* from grasses to the wheat fields by means of the uredospores. Twenty-eight uredo pustules were selected from *Agropyron tenerum*, *A. repens*, *Avena fatua*, and *Hordeum jubatum*, and 230 plants inoculated. From each pustule inoculations were made upon plants of wheat, barley, rye, and oats, and upon the host species from which the rust was obtained. Parallel marks were made upon the leaves with India ink, and the spores placed between them in order to distinguish the results of regular inoculations from accidental infection. There was very little spreading of the rust, however, as the infected leaves were always removed from the greenhouse. The plants were covered with bell jars 24-48 hours, as formerly. Germination tests of the uredospores showed an average viability of 70-80 per cent.

The uredospores of 21 of the 28 pustules caused infection, but showed a decided preference for certain host species. The rust readily infected rye, oats, and the grasses, but not wheat or barley. In fact, the results of the few experiments made seem to show what was anticipated from the two series of infection experiments with aecidiospores, viz., that one form of *P. graminis* is common to *Hordeum jubatum*, *Agropyron tenerum*, *A. repens*, *Avena fatua*, oats, and rye, but is incapable of infecting either barley or wheat. This furnishes little encouragement to those who believe that *P. graminis* is spread to the wheat fields from the barberry bushes or from occasional protected spots, as beneath ice by aid of the native grasses. The data however give no information with respect to the forms of *P. graminis* on wheat or barley, as neither was infected, but in our breeding experiments in 1905 a number of wheat plots were surrounded by a border of barley which was practically destroyed by black rust, and yet there was no visible

evidence that it ever spread to the wheat. Hence it appears that the forms upon these two species are distinct.

The wintering of *P. graminis* as mycelium in plant tissues in North Dakota is extremely doubtful, as there are no winter cereals and the uredo stage does not appear upon the grasses until very late in the spring, when they are quite large. To test the supposition, however, that the fungus might pass the winter in occasional plants under shelter and produce a new outbreak of uredo the following season, I placed heavily rusted plants of *Agropyron tenerum*, *A. repens*, *Hordeum jubatum*, *Elymus virginicus*, and *E. canadensis* in large pots three successive falls (1904-1907) and transferred them to the greenhouse where they remained until summer, but no fresh uredo pustules ever appeared on any of them. Furthermore, in collecting data relative to the appearance of *P. graminis* upon cereals and grasses in the spring of 1905, a piece of low meadow containing *Hordeum jubatum*, *Agropyron tenerum*, and *A. repens*, which was flooded by the city in the winter and used as a skating pond, was carefully observed, but no uredo pustules appeared here until they were found on the grains and grasses elsewhere.

The origin of spring infections has frequently been attributed to over-wintered uredospores, although this is merely a hypothesis. In order to determine with some accuracy the duration of the germinative capacity of the uredospores of *P. graminis*, the following experiments were made. Bundles of rusty straw which had stood in the shock until late in the fall of 1904 were placed on the ground. Others were tied to trunks of trees, and some stored in the attic of one of the college buildings, where the temperature was below freezing but much warmer than the outside atmosphere. Rusty wheat straw was also put in manila envelopes and in test tubes, and these laid in pasteboard boxes on the ground. All the material placed upon the ground was covered by snow the greater part of the winter. To still vary the conditions, test tubes of rusty wheat straw were attached in an inverted position to stakes outside, 2-3 feet above the ground, while packets were buried in ice at the ice house and others kept in the laboratory. From the middle of September to the following July germination tests of the uredospores were made once a week from all these sources. At

the beginning of the experiment, about 10 per cent of the uredospores were viable, but by the end of September this had dwindled to 2 per cent; and only an occasional uredospore germinated in October and none whatever after November 15.

Some confusion arose at first over a fungus whose hyphae emerged from the germ pores, but without showing any conspicuous evidence of its entrance. This fungus proved to be an *Alternaria*, which parasitized many uredospores.

Repeated attempts were made to germinate uredospores from *Hordeum jubatum* and occasionally from *Agropyron repens* which were buried under ice and snow, but always without success. Old uredospores can be obtained in abundance all winter and in early spring on *Hordeum jubatum*, lying between the stem and sheath, but it is practically impossible to find them here on other grasses in winter, although the plants may be buried under ice in low places, as they drop off before winter and are replaced by teleutospores. Hence no uredo pustules were available from other grasses except *Agropyron repens*, whose few winter-borne uredospores would not germinate.

The germination tests show that during the winter of 1904-1905 in North Dakota all or practically all the uredospores of *P. graminis* probably lost their viability, and hence were not the cause of the large amount of black rust in the state the following summer.

The annual reappearance of *P. graminis* in Kansas, Nebraska, and the Dakotas has often been explained by assuming that it passes the winter in Texas and spreads north by means of the wind and growing crops. To obtain data on this point an endeavor was made to catch uredospores of *P. graminis* from the air before any pustules appeared upon the wheat. A post 5 feet high was set in the edge of a wheat plot and a soup dish 7 inches in diameter, containing a small quantity of distilled water, just enough to fill one tube of the centrifuge, was exposed at its top 30-40 minutes. The whole inner surface of the dish was rinsed with the water to collect any spores adhering to its sides. The water was then poured into a tube provided with a tapering bottom, and the débris precipitated by means of a centrifuge. All the particle-bearing liquid was removed from the narrow end of the tube by

means of a pipette, placed upon six slides and examined microscopically. This process was repeated two or three times a week for nearly a month, but no uredospores of *P. graminis* were caught until uredo pustules were abundant on the surrounding wheat.

Further observations were made to determine whether uredospores are commonly borne very great distances by the wind. On a piece of ground one-third to one-half acre in area, which we used for breeding rust-resistant wheat, a rust epidemic was produced every year. This was accomplished by plowing into the soil rusty wheat straw and spraying the wheat repeatedly with aecidiospores of *P. graminis tritici* Erik. and Henn. It should also be mentioned that our original seed, the foundation stock, was obtained from the badly rusted crop of 1904. Hence there were present teleutospores, aecidiospores, uredospores, and probably infected seed. For the present, however, we are concerned only with the fact that rust annually appeared upon these plots in great abundance. In fact it was almost impossible to obtain any plump kernels of wheat from plants grown here. During at least two summers (1906-1907), when these plots were thoroughly covered with *P. graminis*, there was scarcely any rust on the field plots of wheat which lay a short distance north of the infested area and in the direction of the prevailing winds, although the latter passed over the breeding plots, often causing considerable annoyance while I was taking rust notes. The only possible hindrance to the passage of the spores was a few rows of shrubs covering a strip about 10 feet wide, thinly planted and varying from 6 to 8 feet in height, located 20-25 yards from the rust bed. However, there was a road about 20 feet wide running north and south through the shrubbery and along the west edge of the infested area. Hence there was ample opportunity for wind distribution of the uredospores, and former experiments have shown that they were highly viable during the summer of 1906, yet practically no rust appeared upon these neighboring wheat plots. The fact that *P. graminis* does not appear upon wheat in North Dakota in the summer until the plants are nearly 2 feet high, several weeks after the wheat crop is harvested in Kansas and Nebraska,

would also appear to indicate that the spores are not commonly borne very great distances by the wind.

Little or nothing has been done in the past to test the possible infection of sprouting cereals by means of germinating teleutospores from the soil. When buried in moist earth it is not even known whether teleutospores can produce germ tubes or promycelia. A striking fact in connection with the possible infection of seedlings by teleutospores was observed in our field work. Our breeding ground, in which we produced an abundance of rust annually as described above, consisted in 1907 of three parts which, however, were not separated by paths or any visible marks. The whole west half had grown three successive crops of rusted wheat (1905-1907); all the east half except a narrow strip on the north end grew rusted wheat in 1905 and 1907, but produced a crop of flax in 1906; the remainder was in sod until 1907, when it was planted to wheat. The rusty wheat instead of being removed from the ground was always plowed under. The same varieties, except some of Farrer's wheat which was not taken into account, were planted on all three areas at the same dates. The soil was equally level and very fertile. All the wheat was inoculated alike during the spring and summer of 1907, yet shortly before harvest the three parts were separated quite distinctly by lines of rust demarkation, the amount of rust varying with the number of crops of rusted wheat grown upon each area. Whether these results are due to the different quantities of teleutospores in the soil of the different areas or not cannot of course be definitely stated, but they are at least suggestive of the need of further experiments in this direction. If the perpetuation of the wheat rust in the absence of the barberry is due to the teleutospore infection of the germinating seed, variation in the time of seeding might easily account for the annual variability in its prevalence.

The further possibility that rust may be carried in the seed itself is certainly also to be considered. Teleutospores and mycelial fragments of *P. graminis* are often present in abundance in the pericarp of wheat grains, and can frequently be recognized by the appearance of pustules, as will be described later. Early in the spring of 1905 about 60 wheat grains with such contamination

were planted under each of two glass cages provided with cotton ventilators to prevent the entrance of spores from the air. The experiment was afterward repeated in the greenhouse, but rust never appeared on the plants in either case. The conditions however were exceedingly abnormal. The ventilators were entirely too small and the moisture inside the cages was always excessive. While the plants grew rapidly, headed, and blossomed, they failed to set seed both years. Another experiment made in the spring of 1905 appeared to give more favorable results. Wheat was sowed at various dates, some of it quite late. It was all inoculated early and repeatedly with both aecidiospores and uredospores of *P. graminis tritici* Erik. and Henn., the latter being obtained chiefly from the experimental plot of winter wheat of the same source as noted above, but the wheat of every sowing remained nearly free from rust until it began to head, when each in turn became thoroughly rusted. It might be assumed on this evidence that wheat has only a definitely limited period of susceptibility, still very small volunteer wheat plants are often quite rusty in the fall. It is possible to attribute this peculiar behavior to infection through the seed with a long subsequent incubation period in the growing plant, although the possibility of its coming through the soil is not excluded.

The infection of wheat grains with *P. graminis* can often be recognized by the presence of a tiny black spot where the grain separated from the mother plant. When black, this area is generally filled with teleutospores, which can be distinguished in mass with the naked eye or at least with the aid of a hand lens. Such grains are usually shrivelled, but occasionally they remain quite plump. Grains showing a spot of larger area with somewhat irregular boundaries are usually infected with other fungi, as *Alternaria* or *Helminthosporium*, and may not even contain rust. These are the so-called "black-points" mentioned by BOLLEY.⁴

In rusted grains of wheat the pustules are usually most abundant in the thick portion that was formerly attached to the rachilla, but they are also found in other parts of the pericarp, and often lie in the seed coats where they are pressed against the endosperm

⁴ Science, Oct. 21, 1910, p. 1.

or embryo. As many as 10 pustules are sometimes seen in a single section, and nearly all of them are wholly inclosed by the tissues. All about the pustules are masses of rust mycelium, but the hyphae are not confined to these areas. They extend considerable distances from the pustules, and are present in numerous grains in which teleutospores cannot be found.

To obtain further information relative to infection through the seed, badly rusted grains of wheat after germinating from one day to two weeks were studied in the botanical laboratory at Madison, Wis., by means of cytological methods. The seed available was a remnant of former experiments, 4 or 5 years old, and revealed some very interesting phenomena.

Teleutospores in certain pustules, lying in the region of the hilum, were found to be undergoing remarkable changes, resembling the so-called palmella formations of certain filamentous algae. The protoplasts appeared to grow and divide in various directions, often distending the walls until they became quite thin (figs. 5-13). The nuclei, though not well fixed, were present as irregular densely stained bodies. Frequently one or both cells of the teleutospore were still undivided (figs. 1-3, 15), but numerous later stages were present, in which the protoplast had divided one to several times. As a rule, the wall between the two original cells was quite thin and persisted for some time (figs. 5, 6, 8), but occasionally it could not be distinguished (fig. 7). In the latest stages observed the cells became more distinct, often rounding slightly and acquiring thicker walls (figs. 7, 8, 11, 12). A view of the apical end of the teleutospore represented by fig. 12 is shown in a lower focus in fig. 11. In numerous cases the two halves of the former teleutospore finally separated from each other, forming two more or less globular multicellular aggregates (figs. 13a, 18). That these conditions are due to a parasitic mycelium, which has penetrated the teleutospores and completely replaced the protoplasts of the rust, is of course a possibility to be reckoned with. As is seen from the figures, however, direct evidence of the presence of such a parasite is entirely lacking. There is no mycelium outside the rust cells, and no evidence of a gradual absorption and replacement of the rust protoplast by that of a parasite. The subsequent

behavior of the cells will of course show their true nature, but as material is not available for following them through the later stages of germination of the seedling, it seems best to publish as a preliminary account the figures of the stages already found. The importance of their bearing on a possible method of wintering of wheat rust in the absence of the barberry or uredo is apparent.

These peculiar phenomena were not confined to the teleutospores, but were frequently present in the stalk cells (figs. 2, 5, 9, 10), and even in the mycelial region below (figs. 14, 15). Fig. 14 represents a radial section through the base of a pustule, one teleutospore and neighboring stalk cells being included. As is seen, the growing cells of the sorus were associated more or less in groups, but usually interspersed with smaller empty cells. Sometimes they formed dense areas, where it was difficult to determine whether they were of hyphal or teleutosporic origin. Now and then faint outlines resembling distorted, multicellular teleutospores were seen in the mass, but in all probability at least some of the cells arose from the mycelium. Apparently identical cells were found in other parts of the pericarp remote from the pustules (fig. 17). Lying near were filaments composed of similar though usually smaller cells (figs. 16, 17, 19). These however were enlarged portions of a smaller mycelium, all the remaining cells being empty.

Quite separate from the cells just described, fragments and often considerable pieces of what appeared to be living rust mycelium were found mixed with dead hyphae of the rust (figs. 20-22). They were usually in the pericarp, but often lay next to the deepest layer. There were occasional places outside the region occupied by the layer of feeding cells where they passed through into the cells of the scutellum and were found in considerable abundance within 6 or 7 cells of the growing plant itself (fig. 21).

As noted, the fate of the teleutosporic and mycelial cells described above remains for future determination, as my present material contains no later stages. The evident suggestion is that they may serve as growing points for the development of new rust mycelia and the infection of the embryo and seedling.

The possibility for infection of the seedling when the pericarp

of the seed is filled with living rust would seem to depend chiefly on the presence of reserve food for the fungus and the capacity of the hyphae to grow through a few dead cells. The penetration of the dead tissue may and probably does offer some difficulty to the majority of the hyphae, but in some places only a single cell wall of the pericarp intervenes, which could scarcely be looked upon as an absolute obstruction. At any rate, an abundance of mycelium resembling rust was found in the scutellum close to the growing tissue, with apparently nothing to hinder its further progress in that direction.

Whether after infection of the embryo in the manner suggested the rust mycelium might grow with the plant and take on a virulent form at later stages, when it spreads to form pustules, is certainly an interesting possibility. Such a general systemic infection was assumed in ERIKSSON'S mycoplasm theory, and there is some evidence in the general behavior of the rusts as noted above to suggest such a possibility. That such a palmelloid growth of fungal hyphae under peculiar conditions of nutrition is to be expected is abundantly shown by RACIBORSKI'S interesting observation on a palmella-like growth of *Basidiobolus* when placed in media rich in nitrogen. Further investigation of the infection of wheat by rust through the seed will be made when suitable material is obtained.

Summary

1. *Puccinia graminis* passed readily from wheat, *Agropyron tenerum*, *A. repens*, *Hordeum jubatum*, and *Elymus triticoides* to the barberry.

2. Observed facts seem to oppose the theory that aecidiospores and uredospores are carried considerable distances by the wind.

3. Uredo pustules of *P. graminis* appeared upon the experimental plot of winter wheat as early as upon grasses near the barberry bushes, and with one exception were generally present upon the spring wheat earlier than they appeared upon the grasses remote from the barberry.

4. *P. graminis* does not appear to spread to the wheat fields by aid of the grasses. The few experiments made seem to show three distinct biological forms of this fungus: one for wheat, one

for barley, and one for rye, oats, *Hordeum jubatum*, *Agropyron tenerum*, *A. repens*, and *Avena fatua*.

5. Uredospores of *P. graminis* failed to survive the winter of 1904-1905 at Fargo, North Dakota.

6. The wintering of *P. graminis* as mycelium in plant tissues in North Dakota is very doubtful, as shown by the late appearance of the uredo pustules in the spring and the failure of rusted grasses to produce the uredo again after being housed during the winter.

7. The pericarp of rusted wheat grains is frequently filled with rust mycelium and numerous pustules of teleutospores.

8. Teleutospores in some of the germinating grains appeared to be germinating in a palmella-like stage.

9. Pieces of mycelium resembling rust were found in the cells of the scutellum close to the growing plant.

In conclusion, I wish to acknowledge my indebtedness to Dr. R. A. HARPER for aid in the cytological study of the material and in the preparation of the paper.

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EXPLANATION OF PLATE IV

Phenomena appearing in the mycelium and teleutospores of *Puccinia graminis* in rusted grains of wheat during germination:

FIGS. 1, 2.—Teleutospores, showing thin walls and cells preparing for division; fig. 2 also shows an enlarged distorted stalk cell in which a cell wall has been formed.

FIG. 3.—A teleutospore whose lower cell has divided.

FIGS. 4-6, 9, 10.—Early palmella-like stages, showing angular cells with thin walls.

FIGS. 7, 8, 11, 12.—Late palmella-like stages, in which the cells are more or less rounded, thicker walled, and less crowded.

FIG. 13.—A group of teleutospores lying in the edge of a pustule that was sectioned somewhat obliquely.

FIGS. 14, 15.—Radial sections through the base of pustules showing living cells of the sorus.

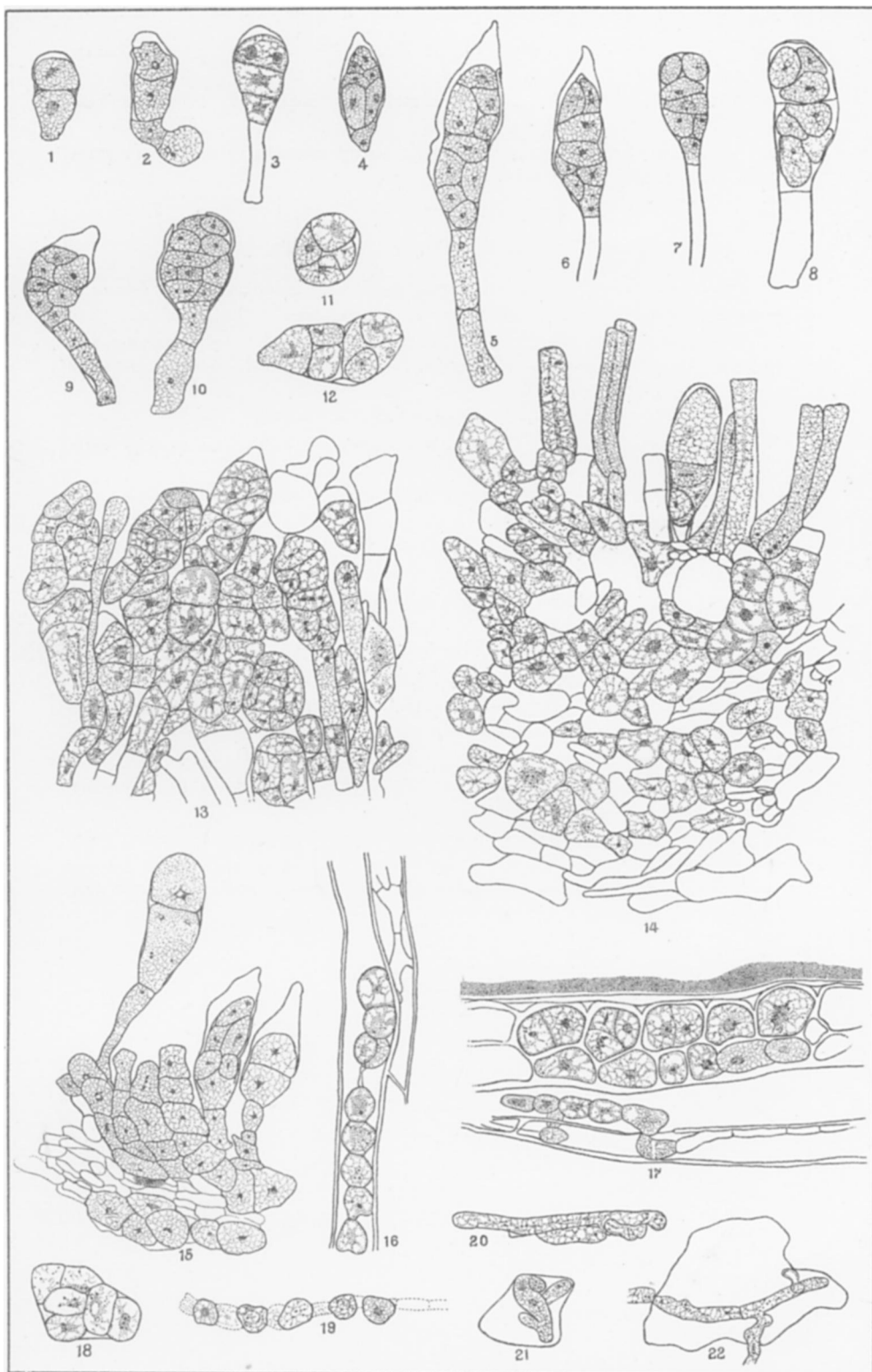
FIG. 16.—Mycelium lying in the pericarp; a few cells were alive and considerably enlarged, while the remainder of the filament was dead.

FIGS. 17, 19.—Dividing cells and living portions of mycelium lying in the pericarp remote from pustules; as in fig. 16, only a small portion of each filament was alive.

FIG. 18.—A group of cells found among the teleutospores of a pustule similar to that represented by fig. 13 (cf. 13a).

FIGS. 20, 22.—Fragments of mycelium found in the pericarp mixed with dead hyphae of the rust.

FIG. 21.—A typical piece of mycelium found in the scutellum within 6 and 7 cells of the radicle.



PRITCHARD on PUCCINIA GRAMINIS